# **Proposal Cover Page**

A proposal submitted for projects in Southeast Florida

Project Title: Investigation of a White "Blotch" Coral Disease Geographic Barrier in the Florida Keys

Principal Investigator(s): Rob Ruzicka and Erinn Muller

Date Submitted: 25 July 2017

Proposal Start Date: 1 October 2017

We, the undersigned, certify that, in the event this proposal is accepted whole or in part, our signatures on this proposal constitute intended acceptance of and compliance with applicable policy, rules, and regulations of the U.S. Environmental Protection Agency.

| ENDORSEMENTS: Submitted by: Principal Investigator | Approved by:<br>Institutional Representative |  |
|--|--|--|
| Signature  | Swam Menphy<br>Signature                     |  |
| Rob Ruzicka  | Susan Murphy                                 |  |
| Typed Name   | Typed Name                                   |  |
| Research Scientist                                 | Administrative Services Manager              |  |
| Title  | Title  |  |
| 100 8 <sup>th</sup> Ave SE                         | 100 8th Ave SE                               |  |
| Saint Petersburg, FL 33701                         | Saint Petersburg, FL 33701                   |  |
| Address  | Address                                      |  |
| 727-242-0557                                       | 727-502-4759                                 |  |
| Phone  | Phone Fax                                    |  |
| Rob.ruzicka@myfwc.com                              | susan, murphy@ myturcom                      |  |
| Email  | Email  |  |

## **PROJECT SUMMARY**

A devastating "white blotch" disease event is currently affecting multiple coral species of Southeast Florida. Unknowns include the type of organism causing the disease, its origination, its extent, its transmission, and how to control/contain it. FWRI, while conducting our currently funded EPA Coral Reef Evaluation and Monitoring Program (CREMP) surveys, has noted that, for the past 3 years, the disease has not spread beyond Tennessee Reef into the Middle Keys although the Upper Keys have been widely and dramatically affected; the same area was the terminus of the giant barrel sponge (Xestospongia muta) die-off in 2012. Coral reef researchers and managers hypothesize that the transmission of the current "white blotch" disease is at least partially water-borne. Initial analyses of the epicenter of the disease outbreak in Miami-Dade County indicate that the initial stages of this disease outbreak was likely contagious and waterborne (Precht et al. 2016). Whether the continuation of the epidemic was transmitted through the water column, against the prevailing currents (northeast to southwest) is currently unknown. This study proposes to answer that question in 2 parts: Do the waters between Alligator Reef and Sombrero Reef serve as a transmission barrier, and if so, why does this barrier occur? To answer these questions, we will collect coral tissue samples, sediment samples, and water quality (bacterial-focused) samples at Alligator Reef (diseased), Tennessee Reef (unknown), and Sombrero Reef (non-diseased). We will attempt to discern differences between the 3 areas using molecular analyses to determine their bacterial communities. Over 10,000 different bacterial operational taxonomic units (OTUs) have been identified living on corals as part of the holobiont; however, we do not definitively know which are beneficial and in what quantities. Focusing on the differences between the 3 reef areas may focus our "type of organism" search in this study and in complementary studies that are utilizing histology and molecular analyses. If the study confirms that the area is a barrier, our partner, Dr. Erinn Muller at Mote Marine Laboratory, will create a spatial/temporal model using the sampling results and available physical oceanographic data to determine whether the barrier is biological or physical in nature or a combination of both.

#### 1. INTRODUCTION

## a. Situation, Need, and Previous Efforts

Disease is recognized as a major cause of reef-building coral mortality and reef degradation. The first reports of coral disease in the Florida Keys and Caribbean emerged in the 1970's. Since that time, worldwide reports have been increasing in frequency and many coral reefs are being decimated. The Florida Reef Tract is currently experiencing one of the largest and longest disease outbreaks on record. Multiple diseases have been reported affecting at least 20 species of scleractinian coral, including primary reef builders and species listed as Threatened under the Endangered Species Act (Table 1). Coral disease outbreaks began in late 2014 offshore the Miami-Dade area, with increased reports peaking in spring 2015 and the outbreak area expanding to the north and south (FDEP disease call reporting). Disease prevalence values as high as 80% of all colonies present at a given site were reported offshore southeast Florida (Miami-Dade, Broward, and Palm Beach Counties) during this time (FDEP reporting). By winter 2015, reports of similar outbreaks were affecting reefs within the Florida Keys National Marine Sanctuary in the northern area of the Upper Keys. Throughout 2016, the affected areas spread north through Martin County and south through the Upper Keys.

The coral diseases reported include white plague, several uncharacterized diseases including "white blotch", white "bleaching band" and an irregular tissue loss that is similar to rapid tissue loss (RTL), which was previously used to describe irregular patterns of RTL on acroporids. Other typical background level diseases have also been reported (i.e., black band, dark spot disease) but they are not as consistent throughout the affected areas as the other diseases previously mentioned.

Initial sampling efforts began in late 2015 targeting diseased coral tissues from several species from the offshore North Miami-Fort Lauderdale area for histological analysis (K. Bohnsack, FDEP; S. Tanner, MDC). Tissues were shipped to FWRI for processing and analysis. In June and July 2016, additional disease outbreaks were reported from CREMP at historical sites in the upper Florida Keys including

Carysfort Reef and Grecian Rocks. The CREMP team, which is part of FWRI, conducted a directed sampling effort at Grecian Rocks targeting both apparently healthy and disease colonies for comparison and collected paired molecular and histology tissue samples for analysis. Prevalence surveys conducted at the time of sampling revealed that 100% of *M. meandrites* colonies, 66.7% of *D. labyrinthiformis* colonies, 53.3% of *M. cavernosa* colonies, 50% of *D. stokesii* colonies, 50% of *P. strigosa* colonies, 42.3% of *S. siderea* colonies, 33.3% of *C. natans* colonies, and 33.3% of *E. fastigiata* colonies were actively diseased or recently dead.



Figure 1. Photos of representative coral species from Grecian Rocks in the upper Florida Keys exhibiting disease lesions. Top left: *D. stokesii* with white plague; top right: *S. siderea* with "white blotch"; bottom left: *M. cavernosa* with "white bleaching band"; *M. cavernosa* with indiscernible disease exhibiting rapid tissue loss.

## b. Objectives

This project proposes to characterize the nature of the geographic barrier to coral disease progression using coral tissue, sediment, and water quality (bacterial) samples. Hypotheses: The study area is a geographic barrier and it is a geographic barrier due to physical oceanographic parameters.

## c. Application, Benefits, Importance

This project will not only help us to understand if and why the current outbreak stopped in this area, but will likely provide predictive capability should any future disease outbreak occur on benthic organisms of the FRT. The results will also inform our ongoing histological and molecular analyses as to the potential organism(s) causing "white blotch." The anticipated results relate to the goals/objective of the FKNMS Water Quality Protection Program as following:

- W.20 Implement Water Quality Monitoring; W.33 Implement Ecological Monitoring
   (coral/seagrass); W.21 Develop Aquatic Resource Predictive Models; W.23 Develop tools for
   pollutants and water quality problems such as loading models and innovative monitoring tools in
   the FKNMS WQPP Action Plan
- Special Study: Investigate Coral Disease Occurrence in South Florida (W.33)
- Supports EPA Strategic Plan Goal 2 'Protecting America's Waters, Objective 2.2 "Protect and Restore Watersheds and Aquatic Ecosystems."

## 2. Methods and Approach

#### d. Description of Major Tasks

First, we will collect coral tissue, sediment, and water samples for molecular analyses at Alligator Reef, designated as a reef within the zone of the outbreak (and therefore preceding the transmission boundary), and at Tennessee and Sombrero Reefs, reefs that lie directly within the proposed boundary area (Tennessee Reef) and a reef projected to be outside of the disease hotspot (Sombrero Reef). The bacterial community of these samples will be analyzed to determine whether there is a significant difference in the

bacterial signature prior to and after the potential geographic barrier. Secondly, we will use past reports of disease occurrence and available hydrological data to model the hydrodynamics around these reefs and the Keys nearby.

#### Field and Laboratory Protocol Development

Field protocols, including those outlined below, will be adapted and developed from: NOAA's established protocols in the Coral Disease and Health Consortium's Field Manual for Investigating Coral Disease Outbreaks (Woodley et al. 2008); George Mason University (GMU) Histology Laboratory protocols for coral sample collection by coring tube (Peters 2012a); Smithsonian Research Station molecular sample preservation (V. Paul personal communication, November 21, 2016). Five replicate plugs of 1 inch in diameter coral tissue will be extracted using a metal coral punch from each coral species. The Sampler will collect the prescribed samples from coral colonies from a maximum of 6 species (i.e., M. cavernosa, D. labyrinthiformis, C. natans, S. siderea, O. faveolata, A. agaricites) under an approved collection permit (i.e., FKNMS-2016-078-A1), photograph the pre-and post-biopsy site, as well as note disease status of the coral colony before sampling. Small 0.5 g of sediment samples will be scooped up using sterile scoopulas and placed within sterile whirlpaks. Finally, one liter of water will be collected within Pyrex containers and processes through a 0.2 micron filter for the collection of bacterial cells. DNA will be extracted from all sample types using the MoBIO PowerSoil DNA extraction kit. Total DNA will be sent to MRDNA labs in Shallowater, Texas for polymerase chain reactions, Illumina high-throughput metagenomics sequencing, and post processing of retrieved OTUs into taxonomic categories. From the metagenomic reads, sequences were binned into OTU's (operational taxonomic units) based on sequence similarity with a 97 % similarity cutoff. OTU's will be classified at all taxonomic levels using BLASTn against a curated database. (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu) The OTU data will then be processed through a series of multivariate (PERMANOVA, SIMPER, NMDS plots) and univariate (ANOVA or Kruskal Wallis tests on each OTU) statistics to determine potential differences in the bacterial communities across the barrier.

## Minimizing Cross Contamination

All sampling will follow established protocols developed for this project to ensure quality and integrity of the samples. Sampling teams will visit sites with no signs of disease first when applicable. Healthy corals will be sampled first and then affected/diseased coral. When sampling affected/diseased coral, unaffected tissues are sampled before disease margin tissues are sampled. All sampling equipment will be sterilized on land before use and placed in separate numbered collection bags for each sample target. Each numbered collection bag, one for each colony to be sampled, contains sterile corer, a pair of nitrile gloves, and pre-labeled Whirl Packs. To minimize cross contamination between colonies, each pair of nitrile gloves will be discarded in a separate designated sealable bag after each colony is sampled. To minimize cross contamination between sites, all collection equipment will be sterilized on the boat in a 5-10% sodium hypochlorite (bleach) solution for 20 minutes, while traveling between sampling sites.

## Data Entry

All field data will be entered in an excel spreadsheet, verified and crosschecked. Excel spreadsheets will be double checked by a different observer to ensure data were entered correctly and any typos corrected. Field data sheets will be photocopied and scanned to ensure there are multiple backup copies of the data. Data are stored on a secure FWC server which is backed up regularly by FWRI IT department. All molecular data will be submitted to the open access data repository, the National Center for Biotechnology Information (NCBI) and Accession numbers will be reported.

#### Statistical Analyses

Rarefaction analysis will be conducted using the vegan package in R (Oksanen et al. 2015) and rarefaction curves will be created for each tissue type using data averaged across all sampling sites in order to visualize the scope of our sequencing. A two-way permutational ANOVA (PERMANOVA) with 9,999 permutations (Anderson 2001) will be used to determine whether there were differences in

microbial communities among sites. PERMANOVA will be conducting using the vegan package in R (Oksanen et al. 2015). Community profiles will be then visualized using non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity index (Kruskal, 1964). NMDS will be calculated using the metaMDS function from the vegan package in R (Oksanen et al. 2015). Non-metric multidimensional scaling (NMDS) will be utilized to compare bacterial OTU communities through collapsing, ranking, and plotting each OTU community position onto a reduced two-dimensional space where type and site within type could easily be compared. In order to identify which sites will be different within each type for bacterial OTU counts, a pairwise permutation multivariate analysis of variance (PerMANOVA) will be conducted using a Bonferroni adjustment. Diversity and species richness will be calculated using the Shannon, Pielou's Evenness, Simpson, and Inverse Simpson methods to compare the bacterial OTU's between sites specific to each community type (i.e. Sediment, Rhizomes, and Leaves). The Shannon diversity index values of the bacterial OTU counts for each site within the community type will be graphed in order to give a visual diversity comparison. After Shapiro and Kruskal-Wallis tests were rejected, a Bonferonni corrected Dunn's test will be used for a non-parametric post hoc comparison using the 'dunn.test' package in R in order to identify any significant differences among the bacterial communities. A dissimilarity index and the dissimilarity percentages of the bacterial OTU community of each site within type will be created using a SIMPER analysis in the R package "vegan". Bacterial OTU's that are found to be dissimilar in 3 or more site comparisons within each community type will be identified. A comparison will then be made between the percentages of the bacterial OTU present within each site. Relative abundance will be conducted for the classes of bacteria within each site specific to each community type. The bacteria classes will then be compared through a Kolmogorov-Smirnov test (K-S test), which will be conducted to identify any specific distributions of bacteria classes among sites within each community type.

## **Modeling disease dynamics**

Collecting information on hydrodynamics of the Florida Keys

Past literature, including peer reviewed publications, white papers, grey literature, and reports will be searched for hydrodynamic process information along the shorelines of South Florida and the Florida Keys. The Florida Current is the prevailing current within this region, flowing from the south west to the east and then north up along the coast of Florida. However, gyre formation, which loops water from the Florida Current into the patch reefs in a counterclockwise direction, is relatively common (Lee et al. 1992). It is hypothesized that these gyres may provide the mechanism for transfer of the putative waterborne pathogen causing "white blotch" outbreak to move south and west, against the prevailing currents.

## Collection of disease occurrence data

Anecdotal evidence of disease presence and absence along the FRT has been observed by several groups including researchers, citizen scientists, and other informative user groups. These data will be gathered into a centralized open source data base, which will be used as the foundation for a spatial disease model. CREMP and SEAFAN reports have been compiled with the Peters and Fogarty (2016) report, however, the continued outbreak and spread requires further increasing the database towards the current disease front. Data will be sorted by disease type and species affected in order to explore the connectivity and spread of the outbreak at different resolutions.

#### Waterborne Disease Model

During the second year of funding, PI Muller will test the hypothesis that the coral disease outbreak is being moved through gyre formation as the waterborne pathogen spreads south and west along the FRT. A spatially explicit Bayesian disease model, similar to Muller and van Woesik 2014 will be applied to the disease occurrence data gathered through the data mining efforts. First, a theoretical disease distribution will be created under the assumption that the disease indeed is transferred as a

waterborne pathogen. Probability of disease occurrence will be weighted based on hydrological information gathered on gyre formation along the FRT. The theoretical model will then be compared with the real data using Bayesian spatial statistics. Comparable spatial patterns of the modeled disease outbreak with the actual disease data will support the hypothesis that this pathogen is waterborne, and transferred through small scale gyres.

## e. Environmental Impact

Coral tissue and skeleton sampling will be conducted by experienced divers and should result in minimal damage to colonies. While this sampling many inhibit colony functions in the short-term, removal of small areas of tissue should not greatly impact colony function long-term. Collection tools will be sanitized onshore between use on colonies to minimize cross-contamination and healthy sites will be visited prior to diseased sites.

## f. Future Efforts

The Coral Reef Evaluation and Monitoring Project (CREMP) is continuing to document the spatial extent of the outbreak in the Upper Keys. Targeted surveys will be conducted in the area of the proposed geographic boundary to confirm the presence or absence of the disease on adjacent reef communities during and the months following this study. Members of the FWRI coral research team are also assisting with follow up surveys to document the extent of mortality at reefs where the outbreak is actively occurring.

#### 3. Project Management

# g. Administration

The co-principal investigators will be Dr. Rob Ruzicka (FWRI) and Dr. Erinn Muller (Mote). FWRI will serve as the grant manager, manage subcontracts, supervise and participate in field operations, write project reports, and present project results. FWRI will subcontract to Dr. Muller to participate in field operations, process samples, conduct data analysis, model results, write project reports, and present project results.

## h. Roles/Assignments and Participation time

#### PRINCIPAL INVESTIGATORS

Richard Robert Ruzicka III MS, Research Administrator I, Habitat/Coral Reef Research, Principal Investigator of CREMP, FWRI Coral Research Project Manager, Contract Manager, Team leader, Benthic Reef Ecologist, Leads *Xestospongia muta* monitoring. Will devote 20% of time at no cost to this project. Erinn Muller, PhD., Staff Scientist, Coral Health and Disease Program Manager, Mote Marine Laboratory. Will devote 320 hours to this project.

## SUPPORTING FWRI CORAL REEF RESEARCH TEAM MEMBERS FOR THIS PROJECT

**Lindsay Huebner, MS,** Research Assistant, Habitat/Coral Reef Research, co-leads CRCP coral recruitment project with Rob Ruzicka. Will devote 14% of time to this project.

**Ananda Ellis, MS,** Biological Scientist II, Habitat/Coral Reef Research, Coral Reef Ecologist, Leads Acropora targeted research. Will devote 14% of time to this project.

**Tiffany Boisvert**, Biological Scientist I, Habitat/Coral Reef Research, Part-time field staff. Will devote 14% of time to this project.

## 4. Support Requirements and Conditions

## i. Cooperation from Other Organizations

This project will require an amendment to an existing FKNMS permit for coral tissue collections.

#### j. Date of Access

The Florida Keys National Marine Sanctuary requires advance notice from permittees wishing to conduct research within Special Activity Areas (SPAs). The FWC Corals Research Program has a long-standing relationship with FKNMS and has maintained compliance for over 20 years.

# 5. Results/Outputs and Deliverables

| PROPOSED TIMELINE - WORK ANTICIPATED TO BEGIN OCT 1 2017 |   |   |
|--|---|---|
| Required Activities/Deliverables                         | FY17/YR 1 (Oct 1 2017 -<br>Sep 30 2018) | FY18/YR 2 (Oct 1 2018 -<br>Sep 30 2019) |
| Programmatic Meetings & Field<br>Collections Planning    | Oct 1 - Mar 30                          |   |
| GIS Analysis of Sampling Effort                          | Oct 1 - Mar 30                          |   |
| Coral Disease Sampling                                   | Apr 1 - Sep 31                          |   |
| Annual Progress Report                                   | Sep 31                                  |   |
| Sample Processing & Analysis                             |   | Oct 1 - Mar 30                          |
| Hydrodynamic Modelling                                   |   | Oct 1 - Sep 31                          |
| GIS Output of Hydrodynamic Model                         |   | Apr 1 - Sep 31                          |
| GIS Output of Water Quality &<br>Bacterial Communities   |   | Apr 1 - Sep 31                          |
| Executive Summary & Final Report                         |   | Sep 31                                  |
| Peer Reviewed Manuscript<br>Publication                  |   | Sep 31                                  |

## **6. Environmental Results**

The outputs and outcomes from the proposed monitoring are described in the methods and approach.

Progress in completing the activities will be summarized in annual reports, presentations at scientific meetings, and an executive summary and final report.

# n. Outputs

# Links to EPA Strategic Plan

- Supports EPA Strategic Plan Goal 2 'Protecting America's Waters, Objective 2.2 "Protect and Restore Watersheds and Aquatic Ecosystems."
  - Characterization of disease status of 20 species of corals at Alligator (currently diseased),
     Tennessee (unknown), and Sombrero (non-diseased) reefs.

- Analysis of coral tissue, sediment, and water samples for bacterial community structure to determine and/or describe the differences across the southern boundary line of the "white blotch" disease event.
- Model of the sample results and obtainable extant oceanographic data to characterize the disease barrier.

#### Outcomes:

- Increased characterization of the "white blotch" disease event along its southern boundary.
- o Increased knowledge as to if the area is a physical disease barrier.
- o Increased knowledge as to why the area is a physical disease barrier.
- o Increased characterization of the spread of the ongoing "white blotch" disease event.

## • Performance Measures

- Successful molecular analysis characterizing the bacterial community of 120 coral tissue samples (6 species, 5 replicates per species, 3 sites = 90 healthy samples, plus 30 disease samples = 120 total coral samples,), 15 sediment samples, and 15 water samples.
- Successful ecological analysis and integration of the above samples to determine if the study area is a barrier to disease spread.
- Successful modelling of the data (sample results and physical oceanography) to hypothesize why the area is a barrier.

# o. Tracking Outputs and Outcomes

The co-PIs will hold quarterly conference calls to track progress and coordinate logistics for sample collection and processing. FWC staff will coordinate with Dr. Muller to provide post-processing and analytical assistance at Mote Marine Institute. Expenditures will be tracked by FWC administrative assistants and reconciled accordingly.

#### 7. Literature Cited

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Lee, T. N., Rooth, C., Williams, E., McGowan, M., Szmant, A. F., & Clarke, M. E. (1992). Influence of Florida Current, gyres and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs. *Continental Shelf Research*, 12(7-8), 971-1002.

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## 8. Budget Information

#### See Attached Excel Spreadsheet

The subcontract to Mote Marine Laboratory (MML) includes salary to cover Dr. Erin Muller's time for sample collection, processing, preparation for sequencing, and conducting the data analysis and reporting out on results. The subcontract to MML and Dr. Muller is inclusive of travel expenses for collections of

samples in the Florida Keys and consumable materials and supplies required for processing of the samples at MML. Consumable field supplies and logistical expenses related to FWRI operations for disease collected are outlined separately from those in sub-award to MML (see attached budget).

#### 9. Biographies and Qualifications

## Rob Ruzicka

Mr. Ruzicka is the Principle Investigator and Lindsay Huebner is the co-Principal Investigator responsible for overseeing the direction and completion of the project. Mr. Ruzicka is Research Administrator for the Habitat/Coral Reef Research group and has served as the CREMP Principal Investigator since 2008. His administrative responsibilities are dually programmatic and scientific. This responsibility includes management of coral reef grants and contracts to assure completion of project goals, preparation and management of annual budgets and planning and coordination of all research activities of the group. Additional responsibilities include recruitment, training and supervision of coral staff (both full-time and hourly), reviewing performance, and ensuring compliance with Commission and FWRI rules, policies, and procedures and Coral/Hardbottom Monitoring Project Quality Assurance Project Plan and the Standard Operating Procedures. Mr. Ruzicka has published multiple papers (both as lead author and a co-author) in refereed journals that have documented the findings of the CREMP project

# Dr. Erinn Muller

Dr. Erinn Muller will be a co-Principal Investigator for the proposed research and her expertise will focus on the molecular analyses of the collected samples. Dr. Muller has over 10 years of direct research and resource management experience, having worked for the National Park Service from 2009 - 2010, and for USGS from 2004 – 2007. Her Ph.D., from Florida Institute of Technology (2011), and her dissertation research focused on coral-disease dynamics of the Caribbean. Her current research is supported by NSF, NPS, EPA, Florida State Wildlife Legacy Grants, and Protect Our Reefs License Plate Grant. Previous publications including next generation sequencing have characterized the micriobiome of anemones off a

natural CO2 seep in Italy (Muller et al. 2016), and the members of black band disease under ocean acidification conditions (Muller et al. 2017).

## 10. Programmatic Capability and Past Performance

FWRI has been conducting CREMP activities since 1996. During this time FWRI CREMP has received funding from both EPA and NOAA to complete annual monitoring. In 2004, the National Park Service (NPS) provided funding to establish additional monitoring sites within the Dry Tortugas boundary. CREMP was also expanded in 2003 to include reefs in Southeast Florida (SECREMP) which has added another 23 sites. SECREMP is funded separately from CREMP in the Florida Keys. In addition, NOAA CRCP is currently funding the juvenile coral and settlement project in the Florida Keys. The coral reef group at FWRI has also been awarded funds to assess benthic recovery in the Gulf of Mexico after a severe harmful algal bloom in 2005. A list of the awards is as follows:

- 1) <u>Coral Reef Evaluation and Monitoring Project-Florida Keys National Marine Sanctuary</u> (FKNMS)-Award X7-95447709 (Ongoing). For this project, biannual progress reports were submitted. Executive Summaries and oral presentations at the WQPP Steering Committee Meeting were provided annually. Performance measures (e.g. percent coral cover) were provided to the EPA grant administrator. Deliverables and reporting for this project are in good standing.
- 2) <u>Dry Tortugas National Park (DRTO) Long Term Coral Reef Monitoring & Assessment Project</u>

  <u>-Cooperative Agreement P13AC01267 (Ongoing)</u>. For this project, biennial reports were submitted. Field reports, and presentations to the NPS prepared as needed. All data have been provided to the NPS grant administrator. Deliverables and reporting for this project are in good standing.
- 3) Southeast Florida Coral Reef Evaluation and Monitoring Project Contract RM143 (Ongoing). For this project, quarterly reports are submitted. Annual Reports are also submitted. All data has been delivered the FDEP contract manager. Deliverables and reporting for this project are in good standing.

- 4) <u>Investigating How Coral Recruitment and Juvenile Survivorship Varies Along the Florida Reef</u>

  <u>Tract NOS Agreement Code: MOA-2015-047 (Ongoing).</u> For this project, annual reports are submitted that summarize field efforts and data collected. Data Summaries and presentations are prepared for the NOAA grant officer as needed. Deliverables and reporting for this project are in good standing.
- 5) <u>Gulf of Mexico Benthic Mortality and Recovery Project Award NA06NMF4410082</u> (<u>Closed</u>). This project has been completed. Quarterly and final reports were delivered on time and all deliverables approved by the granting agency.

# 11. Voluntary Cost Share/Match and other Leveraged Funds

The Principal Investigator (Rob Ruzicka) and additional Coral Reef Evaluation and Monitoring Program (CREMP) staff will assist all efforts at no cost to this project. The value of the FWC 26' Twin Vee vessel, FWC vehicles, and trailer (estimated value of \$480/day X 12 field days) will also be dedicated to this project for a total of \$5,760. The funds provided by the EPA WQPP to the CREMP are highly complementary to the programs carrying out assessments and investigative research related to the disease outbreak. CREMP is providing real-time updates on the spatial extent and severity of the outbreak throughout the Florida Keys.

#### 12. Partnerships

FWC/FWRI has a long history of partnerships in FL. Specifically tied to this outbreak, FWRI has worked alongside multiple agencies (FL Department of Environmental Protection and NOAA) and research institutions (Smithsonian, Mote Marine Laboratory, University of Florida, Florida International University, Nova SE University, Texas A&M) to coordinate sampling efforts and distribution among interested parties. State of Florida Marine Resources Conservation Trust Fund supports the salary of the FWC/FWRI Principal Investigator that manages CREMP operations.